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Infectious Bronchitis in Fowls

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INTRODUCTION

Infectious bronchitis is considered a relatively new disease of fowls in the United States. There are, however, several references in the literature which would lead one to believe that the disease has existed among poultry in this country for a long time. Salmon,⁽¹⁾ ² in 1899, in discussing ordinary bronchitis of fowls, the cause of which he attributes to "exposure to dampness, to colds, to drafts of cold air and sudden and extreme changes in temperature"; states further that, "occasionally the malady increases in intensity, the plumage becomes rough, the wings droop, the breathing is labored and difficult, the bird frequently opens its beak in order to obtain sufficient air, it is sleepy, torpid and exhausted. In such severe forms of the disease a large proportion of the affected birds die." Robinson,⁽²⁾ in 1917, described a form of bronchitis as follows: "The fowls had occasional paroxysms of coughing which sometimes exhausted them for a time, but most would quickly regain vitality and seem alright until the next attack of coughing, then all at once it would be noticed that coughing had ceased and the bird was permanently well again." Kaupp,⁽³⁾ in the same year, after describing bronchitis due to exposure to drafts and dampness, stated, "there is a bronchitis and tracheitis found among fowls that is apparently contagious." This appears to be the first suggestion that an infectious or contagious type of bronchitis might exist. Pearl, Surface and Curtis,⁽⁴⁾ in 1920 stated that "in ordinary and chronic forms the birds usually recovered, but in the more severe forms a large percentage of the affected birds die." Ward and Gallagher⁽⁵⁾ in the same year in discussing the symptoms of bronchitis stated that, "in severe cases the fowl displays a marked difficulty in breathing, the neck is more or less straightened out to facilitate the passage of air, the breathing is noisy and the fowl may gasp in a manner similar to that shown by a chicken affected with the gapes. The beak may be opened and a mucus discharge is given off through the mouth. The bird is observed to make efforts to dislodge this by shaking the head."

¹ Veterinarian in charge of Petaluma poultry disease laboratory.

² Superscript numbers in parenthesis refer to 'Literature Cited,' at the back of this publication.

Although these symptoms are given as those of bronchitis resulting from the extension of inflammation from the mouth and nasal chambers or from exposure to cold drafts and dampness, they provide a remarkably good description of symptoms of infectious bronchitis.

In 1924, Gwatkin,⁽⁶⁾ reported studies of what he termed a very acute type of chicken-pox or avian diphtheria in which the trachea became filled with sticky, bloody exudate and clots of blood. He concluded that the causative agent was the filterable virus of chicken-pox. Further studies by this same investigator⁽⁷⁾ reported the following year, however, failed to confirm these conclusions and the disease is discussed as tracheitis of unknown origin. The first definite report of the existence of infectious bronchitis appears to be that of May and Tittsler,⁽⁸⁾ in 1925, concerning an outbreak of tracheo-laryngitis in a Rhode Island flock in 1923. These investigators recognized the disease as being distinct from ordinary bronchitis and demonstrated that it could be transmitted by intratracheal inoculation of healthy fowls with exudate from the trachea of diseased fowls. They failed, however, to demonstrate the nature of the causative agent and stated that three of the four outbreaks observed appeared to have been brought on by exposure to cold.

During 1924–1925 the disease became prevalent in many parts of the country and was studied by Eriksen⁽⁹⁾ of Missouri, Hinshaw⁽³⁾ of Kansas and Beach⁽¹⁰⁾ of California. Eriksen⁽⁹⁾ found the disease occurring principally in poultry feeding stations and following poultry shows. He considered it to be due to non-specific causes. Hinshaw³ also found the disease more prevalent in feeding stations than on farms and pointed out that birds became affected following exposure to cold during transport in open trucks or poultry cars. He found the disease to start as a typical bronchitis and accordingly termed it “infectious bronchitis.” This name has since come into general use. In bacteriological studies by Hinshaw of the tracheal and bronchial exudate in sixty-eight birds, *Pasteurella avicida* and an unidentified *diplococcus* were frequently isolated. It was not shown, however, that these organisms were of etiological importance. He concluded that exposure and undernourishment are important factors in the causation of the disease. Transmission of the disease to healthy fowls was accomplished by intratracheal inoculation and in one case by intraabdominal inoculation. Beach⁽¹⁰⁾ studied the disease both in fowls on poultry farms and in market fowls shipped in carload lots from the Middle West. He found no evidence that exposure, under-

³ Personal communication from W. R. Hinshaw.

nourishment or similar factors were associated with the occurrence of the disease on the poultry farms. Bacteriological studies of fowls that had died from the disease on poultry farms gave uniformly negative results. *Pasteurella avicida* was isolated from some of the market fowls originating in the Middle West. Intratracheal inoculation with chicken-pox virus in some instances produced lesions similar to those of infectious bronchitis. Transmission of the infection to healthy fowls was successful with thirteen of fourteen birds inoculated intratracheally and with three of nine birds inoculated intraabdominally. Intravenous inoculation produced no infectious bronchitis but did cause the death of four of ten birds from *Pasteurella avicida* infection. Intraabdominal and intravenous inoculations with sterile filtrates of tracheal exudate emulsions failed to produce the disease.

The only reference to the occurrence of a disease of fowls closely resembling infectious bronchitis outside of the United States and Canada that has come to the writer's attention was that of Doyle⁽¹¹⁾ in 1927, concerning an outbreak in England of what he termed "a hitherto undescribed disease of fowls due to a filter passing virus" and which he designated "New Castle disease." There were sufficient differences between New Castle disease as described by Doyle and infectious bronchitis, however, to indicate that they were distinct diseases.

The preceding includes all of the important references in literature to infectious bronchitis. It is seen that the principal results of these studies have been to establish that the disease is infectious and that it probably is distinct from other known diseases of poultry. In the following pages are given results of studies of infectious bronchitis made at the California Agricultural Experiment Station during the past three years. These studies include laboratory experiments and observations of more than three hundred flocks comprising two hundred thousand fowls.

OBSERVATIONS ON THE OCCURRENCE OF THE DISEASE

Course and Mortality.—In most of the outbreaks that were studied, the disease appeared very suddenly and spread rapidly through the flocks. The number of fowls that became infected was rarely less than 50 per cent, not uncommonly was as high as 90 per cent, and in a few instances included all fowls in the flock. The height of infection in the flocks was usually reached by the seventh or eighth day after which it rapidly subsided and in most instances had entirely disappeared

within fourteen to twenty-one days after the onset of the disease. In a few flocks the disease spread slowly. In such instances the number of new cases continued to appear during a period of five to six months. Also in some flocks following an acute outbreak, many of the birds continued to exhibit evidence of inflammation of the nasal passages and eyes. This was probably due to the types of infection that commonly are associated with inflammation of these parts rather than to the "virus" of infectious bronchitis. The mortality usually has been from 10 to 20 per cent of all fowls in the flock but in some instances has been less than 5 per cent or as high as 60 per cent.

Comparison of the range of incidence of infection with that of mortality showed that recovery of many of the infected fowls took place. The percentage of infected fowls to recover, however, varied greatly in different flocks and, therefore, a high or low incidence of infection was not always accompanied by a correspondingly high or low mortality.

In illustration of the variation in extent of the infection and mortality from infectious bronchitis the following descriptions of outbreaks in several flocks are given.

Flock 1 consisted of 850 pullets of unusually good quality, ten months old. Not more than 3 or 4 per cent of the birds became affected at a time. The total loss from death was 8 birds. Egg production, however, which was approximately 70 per cent when infectious bronchitis appeared was materially reduced for a period of four weeks.

Flock 2 contained 850 pullets about eight months old and of good quality and was laying at the rate of about 50 per cent when infectious bronchitis started. The disease affected but a small percentage of the fowls; caused the death of only one; and did not seriously affect egg production.

Flock 3 consisted of 800 ten-months-old pullets. During the outbreak of infectious bronchitis in this flock approximately 90 per cent of the birds became affected and slightly more than 20 per cent of them died, during a period of twelve days. Egg production was decreased from 45 per cent to less than 5 per cent.

In flock 4 which consisted of 600 birds nine months old, infectious bronchitis caused a loss of 372, or 62 per cent of the fowls within nine days.

Flocks 5 and 6 each contained approximately 1300 pullets fourteen weeks old. The total losses caused by infectious bronchitis were 8 and 25 birds respectively.

In contrast to the relatively insignificant mortality in these two flocks, is that in flock 7 which contained 11,000 chicks eight weeks old. The loss caused by infectious bronchitis exceeded 1800, or 16.3 per cent of the chicks within two weeks time.

Symptoms and Morbid Anatomy.—The first indication of the presence of infectious bronchitis that has usually been observed is lachrymation from one or both eyes. This was frequently preceded by decrease in appetite and also, in the case of laying hens, sometimes by a decrease in egg production. The typical manifestations of respiratory distress by from one to several fowls of a flock appeared within twenty-four hours. These are as follows: At inspiration the head is elevated, the neck is extended, the beak is opened wide, and the intake of air is usually accompanied by a loud wheezing sound (fig. 1). During the expiration the head is lowered, oftentimes until the beak rests on the breast (fig. 2). Many fowls assume a sitting posture and their eyes are kept closed. Violent coughing, by means of which masses of clotted blood or mucus may be expelled from the trachea is very commonly observed. When the trachea or bronchi become nearly or entirely occluded with exudate or clotted blood, spasms of coughing and convulsions occur and death quickly ensues. In some flocks, in addition to the above symptoms, many of the birds showed a mucus discharge from the nostrils, distention of the nasal sinuses with exudate, or the formation of a caseous mass within the eyelids as in common colds or roup. Graham⁽¹²⁾ has recently described two distinct types of the disease; a dyspneic and a toxemic type. The writer has never observed the toxemic type unless those fowls that have become so exhausted in their efforts to get sufficient air that they remain sitting with closed eyes much of the time might be considered as exhibiting symptoms of toxemia.

In the early stages of the disease there was hemorrhage of varying degrees into the lumen of the bronchial tubes and trachea. The blood after coagulation was in some cases expelled in a spasm of coughing, in some cases completely obstructed the passage of air, thereby causing rapid death, and in some cases collected on the walls of the trachea as a thin film or long thread but did not entirely cut off the air supply. Such a film or thread of clotted blood sometimes extended the entire length of the trachea. The clotted blood was rapidly transformed into a yellowish cheesy mass which in some instances could be expelled but in a considerable number of cases it collected in the superior larynx in a mass so large that it completely occluded the opening into the trachea and caused death of the fowl. The degree of inflammation

in the bronchi and trachea varied from slight to very severe. No lesions were observed in any of the other organs or tissues of the body.

Hinshaw stated, "The examination of the mouth reveals nothing in the early stages but in the later stages diphtheritic ulcers are nearly always present. . . . In many chronic forms avian diphtheria develops and a few birds may recover." Beach⁽¹⁰⁾ does not confirm these statements but says, "In our experience, however, the fowls exhibiting respiratory symptoms only, either die within two to three days or make complete recovery. In fact our observations lead us to believe that recovery was not infrequent when there was no serious involvement of either the eyes or nasal sinuses." The writer's observations indicate that a high percentage of fowls recover spontaneously from the infection and rarely if ever does a chronic form of the disease develop. The diphtheritic ulcers observed by Hinshaw have never been encountered except in such cases in which the flocks affected with infectious bronchitis were also affected with chicken-pox.

Other morbid anatomical changes described by Hinshaw such as, "Catarrhal to hemorrhagic enteritis," and "Caseated masses of pus found in all internal parts of the body," have never been observed by the writer.

Animals Susceptible.—Natural infection with infectious bronchitis has been observed in several species of birds other than the common fowl. These include turkeys and ducks and such free-flying birds as sparrows, blackbirds, quail and pigeons which frequent poultry yards. In case of the wild birds diagnosis was made by finding typical lesions of the disease in birds found dead on poultry farms on which the disease was present. The various breeds of chickens seem to be equally susceptible. Attempts to infect guinea pigs and rabbits by inoculation with tracheal exudate have been unsuccessful.

Age of Flocks Infected.—Infectious bronchitis has occurred in birds varying in age from three weeks to three or four years. If the incidence and severity of outbreaks can be taken as guides, the fowls between the ages of four and eighteen months are most susceptible. Although young chicks have frequently contracted the disease, more often they have remained healthy when subjected to exposure from infected flocks of older birds on the same premises. Birds two years old and older have appeared to have a marked resistance to the infection. On the larger poultry farms on which it is common practice to have at the same time birds of various ages, such as, broods of chicks, flocks of developing pullets and laying hens, the latter flocks consisting of birds one, two and three years old, the infection has been known



Fig. 1.—Infectious bronchitis, during inspiration.

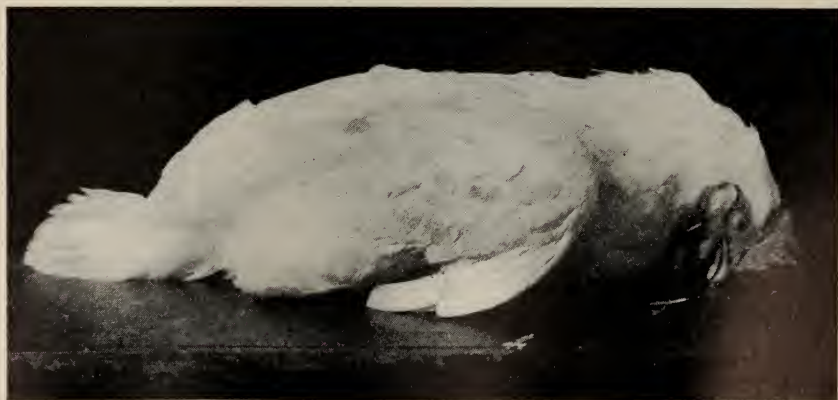


Fig. 2.—Infectious bronchitis; in a state of exhaustion.

to occur extensively among the pullets and yearling hens, to a less extent in the two-year-old birds and not at all among the young chicks and older hens.

Factors Favoring Outbreaks.—As previously mentioned, some investigators have expressed the opinion that exposure to cold and dampness and undernourishment are important factors in the causation of infectious bronchitis. The writer, however, has not observed that the disease has been more prevalent on poultry farms in California during cold and damp weather than when the weather was dry and warm. The incidence of the disease has been greater during the fall and winter months than at other times of the year but this, especially during the fall months was not associated with inclement weather. It is, of course, possible that had the weather conditions been more severe the mortality would have been greater.

Undernourished flocks have not been attacked more frequently than flocks that were properly fed and cared for. It has been observed, however, that the mortality was greater among birds that were undernourished than among birds in good condition. This is contrary to the observations of Beach⁽¹⁰⁾ that the disease seems to attack principally the better birds of a flock. It has also been observed that an unusually high mortality is liable to occur in flocks that are heavily infested with intestinal round worms or with body lice. An exceptionally large number of deaths has also been frequently observed among flocks of pullets or yearling hens that were in a good state of nutrition and laying heavily at the time the disease appeared. The losses in such cases were aggravated by the occurrence of prolapses of the oviduct and rupture of yolks into the abdominal cavity.

INFECTIOUS BRONCHITIS NOT RELATED TO CHICKEN-POX

The results of some of the earlier studies of some investigators suggested that the virus of chicken-pox might be present in the tracheal exudate of fowls that had died from infectious bronchitis and that this virus might be a factor in the etiology of the disease. To provide more information on this point tracheal exudate from three different sources was applied to a scarified surface on the combs of 10 cockerels. None of these cockerels developed either the lesions of chicken-pox on the comb or any of the symptoms of infectious bronchitis. Intratracheal inoculation of other birds with the same material, however, produced definite symptoms of infectious bronchitis. During subsequent experiments with infectious bronchitis, tracheal

exudate that was being used for inoculation by various ways, was applied to a scarified surface on the comb of one or two cockerels as a check on the possibility of the material being contaminated with chicken-pox virus. In no case were the lesions of chicken-pox produced.

Furthermore, Beach⁴ in his experiments reported the occurrence of infectious bronchitis among experimental birds which were known to be immune to chicken-pox. He stated that:

- “1. There were thirty-five cases, with twenty-five deaths from infectious bronchitis among birds known to be immune to chicken-pox.
2. There were forty-four cases, with but twenty-six deaths from infectious bronchitis among birds vaccinated but not yet immune to chicken-pox.
3. There were three cases and deaths among birds with active chicken-pox lesions from artificial infection.
4. The above comprises all of the cases of infectious bronchitis which occurred among more than one thousand birds that were used in the chicken-pox vaccine experiments and tests. The irregularity of its appearance and the number of cases occurring among birds known to be immune to chicken-pox indicate that there is no relationship between infectious bronchitis and chicken-pox vaccination.”

Infectious bronchitis has been observed by the writer in flocks in which recovery from an attack of chicken-pox had occurred some months previous. And flocks which had recovered from infectious bronchitis have been known to become infected with chicken-pox some months later.

Bacteriological Studies of Infectious Bronchitis.—Aerobic and anaerobic cultures have been made from the tracheal exudate and internal organs from a large number of birds which have died from infectious bronchitis. The culture media used for aerobic cultures included broth, nutrient agar, glucose agar, serum agar, fresh and cooked blood agar, blood serum and Dorset's egg medium. The blood agar in some cases was not heated after the addition of defibrinated blood, in some cases was heated at thirty, seventy and ninety degrees centigrade, and in some cases contained whole blood which had been treated with saponin. The nutrient broth used was adjusted to pH

⁴ Personal communication from J. R. Beach.

values varying from pH 6. to pH 8.5. Anaerobic cultures were made in media containing one per cent dextrose and in pieces of sterile muscle tissue and of brain tissue of chickens. These cultures were in some instances covered with a layer of oil and in others were incubated in anaerobic jars in which the air had been replaced by hydrogen. Broth containing one per cent dextrose, lactose, sucrose, maltose or mannite and Andrade's indicator were used in the identification of organisms obtained in the cultures. No attempt was made to identify them by means of serological tests.

The cultures made from the heart blood, liver, spleen and kidneys of birds which had died from infectious bronchitis for the most part remained sterile. In a few instances *Eberthella sanguinaria* and in two instances *Salmonella pullorum* were isolated from the spleen and liver. Of 30 birds which were obtained from one flock *Eberthella sanguinaria* was isolated from the liver and spleen of 5. The birds in this flock were about eighteen months old, and the mortality in the flock had been high for several weeks previous to the outbreak of infectious bronchitis. The mortality due to infectious bronchitis was approximately 35 per cent. *Pasteurella avicida* has been found in the heart blood, liver and spleen in some cases. In one flock of three-year-old hens, in which the mortality during the outbreak of infectious bronchitis exceeded 40 per cent, *Pasteurella avicida* was isolated from the internal organs of 8 of 22 birds which died in one day. Cultures from the tracheal exudate were usually sterile but *Pasteurella avicida* was sometimes present. Cultures made from 18 birds, 3 from each of six flocks, were all sterile. Blood smears and smears of tracheal exudate were examined microscopically by means of the dark field and after staining with Wright's blood stain. The blood appeared to be normal.

TRANSMISSION EXPERIMENTS

Numerous trials have been made to reproduce the disease by the inoculation of healthy fowls by various methods with the tracheal exudate and organs of fowls that had died from infectious bronchitis. The tracheal exudate used for these trials was collected from the trachea of birds which had died from an acute attack of the disease and consisted of coagulated blood mixed with varying amounts of mucus. The exudate was triturated into a uniform pasty mass, then suspended in sterile physiologic saline. The fowls used were for the most part normal cockerels about twelve weeks of age. The results are as follows.

Intratracheal Inoculations With Tracheal Exudate and Organs.—

In a number of trials little difficulty was experienced in reproducing the disease in normal cockerels either by means of a cotton swab saturated with a suspension of the exudate or by means of a pipette. It was found, however, that there was greater certainty of a successful inoculation when the mucous membrane of the trachea was injured by the inoculation procedure. The predominant lesion in such cases tended to be the formation of a tough, yellowish, false membrane oftentimes in the shape of a cylindrical cast extending the entire length of the trachea. Considerable variation was observed in the pathogenicity of exudate from different sources. Differences in pathogenicity were also observed between exudates consisting largely of clotted blood and those containing considerable caseous material, the former being uniformly more pathogenic than the latter.

Intratracheal inoculations with emulsions of liver, spleen, kidneys and intestinal contents of dead fowls uniformly failed to produce symptoms of infectious bronchitis. The same was true of inoculation of two fowls with caseous exudate removed from the eye of a fowl with involvement of that organ.

Inoculation by Introducing Tracheal Exudate into the Crop.—

Heavy suspensions of tracheal exudate from two sources were introduced with a pipette into the crops of 8 normal cockerels in amounts varying from 1 to 5 cc. In no case were symptoms of infectious bronchitis produced. The production of infectious bronchitis in other cockerels that were inoculated intratracheally with the same suspensions provided evidence that they contained the causative agent of the disease.

Subcutaneous Inoculation with Tracheal Exudate.—Suspensions of tracheal exudate from three sources were injected subcutaneously in 1 cc amounts into 6 normal cockerels. None developed symptoms of infectious bronchitis. The positive results of intratracheal inoculation of other cockerels with the same exudates showed that they contained the causative agent of the disease.

Intravenous Inoculation with Tracheal Exudate.—Each of 6 cockerels was inoculated intravenously with 1 cc of a suspension of tracheal exudate. All of the birds died within twenty-four hours. Cultures made from the heart blood, liver, spleen and lungs were negative. The trachea was normal in appearance. These birds did not develop symptoms of infectious bronchitis during the short interval between inoculation and the time of their death. Death undoubtedly resulted from obstruction in the vascular system to the circulation of the blood by

particles in the material injected. In a second trial 6 birds were inoculated intravenously with 0.1 cc of the supernatant of centrifugalized trachea exudate. All birds remained normal. Control cockerels inoculated intratracheally with the same material developed definite symptoms of infectious bronchitis.

Intratracheal Inoculation with Filtrates.—Portions of tracheal exudate, heart blood, lungs, liver, spleen and kidneys of fowls that had died from infectious bronchitis were separately mascerated, suspended in physiologic saline, and passed through a Seitz filter. Cultural tests showed the filtrates to be sterile. Two cockerels were inoculated intratracheally with a pipette with 0.1 cc of each of the six filtrates. All birds remained healthy during the period of thirty days they were kept under observation.

Another suspension of tracheal exudate in physiologic saline was centrifuged for twenty minutes, the supernatant liquid decanted and divided into four equal portions and each portion passed through a different type of filter. The types of filters used were Seitz, Mandler ($\frac{5}{8}$ by $2\frac{1}{2}$ in.), Berkfeld "W" ($\frac{5}{8}$ by $2\frac{1}{2}$ in.), and Chamberland "F" (25 by 200 mm). Culture media inoculated with the filtrates remained sterile. Three cockerels were inoculated intratracheally with a pipette with 0.1 cc of each filtrate. No symptoms of infectious bronchitis developed in any of the 12 birds. The virulence of the material before filtration was demonstrated by production of infection in cockerels that were given intratracheal inoculation.

Beach in 1927 summarizes the results of inoculations with bacteria-free Seitz-filtrates of suspensions of tracheal exudate as follows:

1. Nineteen birds were inoculated intratracheally; 11 sickened in three to eight days; 4 died in four to thirteen days after inoculation.
2. Four birds were inoculated intraperitoneally; 3 sickened in seven to nine days; 2 died in nine to ten days after inoculation.
3. Five birds were inoculated intravenously; 3 sickened in six to seven days; 1 died on eleventh day after inoculation.
4. Five birds were inoculated subcutaneously; none became sick or died.

THERAPEUTIC TREATMENT OF INFECTED FOWLS

Several trials were made to determine the therapeutic value of the injection of antiseptic solutions into the trachea of fowls with infectious bronchitis. The preparations used were mercurochrome,

argyrol, tincture of iodine, acriflavine, cresatin and a solution of sodium hypochlorite. Varying amounts of these were introduced into the trachea of the infected birds with a pipette. The results, as given in table 1, show that the administration of antiseptic solutions, even in such small amounts as 0.2 cc to fowls with infectious bronchitis is attended with considerable danger of causing death within 20 minutes. The results further show that the number of fowls that survived the period immediately following the administration of the antiseptic and recovered was no greater than the normal expectancy of recovery without treatment and, therefore, that no benefit was derived from the treatment.

TABLE 1
RESULTS OF INTRATRACHEAL ADMINISTRATION OF ANTISEPTIC SOLUTIONS

Treatment	Dilution	Dosage	Number of birds treated	Number of birds dying within 20 minutes after treatment	Number of birds dying within 24 hours after treatment
Mercurochrome.....	2 per cent aqueous solution.....	0.2 cc	12	3
		0.5 cc	35	11	17
		1.0 cc	21	17	4
Argyrol.....	10 per cent.....	0.2 cc	36	4	27
		1.0 cc	16	5
Tincture of iodine.....	50 per cent.....	0.2 cc	30	5	23
		1.0 cc	12	5
Acriflavine.....	1 per cent.....	0.2 cc	12	2	5
		1.0 cc	50	14
Cresatin.....	Undiluted.....	0.1 cc	18	16
		0.2 cc	9	9
		1.0 cc	5	5
Sodium hypochlorite solution, .04 per cent available chlorine.....	1 per cent.....	0.2 cc	12	2	5
		1.0 cc	36	1	18

FLOCK TREATMENT WITH VAPORIZED DISINFECTANTS

Several tests were made in the spring of 1929 to determine the value of treating infected flocks by vaporizing coal-tar disinfectants in the poultry houses at night while the birds were on the roosts. For this purpose an electrically heated water bath was improvised with a thermo-regulator to maintain a temperature of 180 degrees centigrade. All openings in the houses that were not provided with means for closing were covered with burlap.

One flock treated consisted of 850 hens. Treatment was started immediately after the observance of two cases of the disease. An undiluted coal-tar disinfectant was vaporized in the house for four hours each night and the treatment continued for eleven days after the infection appeared in the flock. The mortality from infectious bronchitis during the eleven days amounted to 48, or 5.6 per cent of the flock. A record of the number of fowls that became infected was not obtained. Egg production rapidly declined from 450 per day at the start of the outbreak to 230 per day ten days later.

TABLE 2

MORTALITY RECORD IN FLOCK OF 2,000 PULLETS TREATED WITH VAPORIZED COAL-TAR DISINFECTANT

Day	Daily mortality	Mortality distribution by pens							
		pen 1	pen 2	pen 3	pen 4	pen 5	pen 6	pen 7	pen 8
1	4	4							
2	8	6	2						
3	16	12	0	4					
4	12	10	1	1					
5	3	2	0	1	0				
6	8	3	2	3	0	0	0		
7	18	0	2	6	3	7	0		
8	13	0	3	6	1	2	1	0	
9	20	1	1	8	1	3	5	0	1
10	37	1	0	2	2	7	11	9	5
11	29	0	2	1	2	2	12	4	6
12	38	3		1	0	9	8	9	8
13	34			1	2	4	7	12	8
14	32			1		5	2	18	6
15	17					2	1	8	6
16	5						0	3	2
17	3						0	2	1
18	3						1	1	1

Fifty-eight of the affected fowls were removed to the laboratory where they were divided into two lots of 29 each which were placed in separate small houses. One lot was exposed to coal-tar disinfectant vapor for ten hours daily until all had died or recovered. The other lot was untreated. The mortality among those treated was 2 birds and among those not treated was 5 birds. Since both the numbers of birds and the mortality in both groups were small this difference cannot be regarded as significant.

Another flock consisted of 2000 pullets, seven months old. The birds were in 8 groups of 250 each, all in one long house. The infection appeared among the birds of one pen and within twenty-four

hours the symptoms of the disease had developed in the birds in another pen. The infection spread to the birds of every pen in the house within nine days. Electric heaters were placed in each pen and all openings in the building were covered with burlap. The coal-tar disinfectant was continuously vaporized in the pens from the time symptoms were first observed until the disease had disappeared. In some instances, canvas was hung before the roosts to confine and concentrate the vapor in the roosting compartments.

The mortality in the different pens is tabulated in table 2. A record of the number of fowls that became infected was not obtained.

In table 2 it is seen that the total mortality in the second flock from infectious bronchitis was 300 fowls or 15 per cent of the flock. This is no smaller than the mortality that has occurred in many flocks to which no treatment was given and, therefore, indicates that the treatment was of little, if any, benefit. This point is further emphasized by comparing the number of deaths in the different pens. This varied from 11 birds or 4.4 per cent of the flock in pen 4, to 66 birds or 26.4 per cent of the flock in pen 7. The former is considerably less and the latter is considerably greater than the average mortality resulting from an outbreak of infectious bronchitis. The mortality records also show that the course of the outbreak in the different pens varied from seven to twelve days. This provides a good illustration of the time that an outbreak of bronchitis will usually require to run its course.

IMMUNITY PRODUCED BY AN ATTACK OF INFECTIOUS BRONCHITIS

In the course of field studies of infectious bronchitis it was observed that the disease rarely occurred more than once in the same flock although many flocks that had passed through an outbreak became subsequently exposed to the infection by the occurrence of the disease in other flocks on the same premises. A few instances of recurrence came to the writer's attention but in no case could it be determined that the same individuals had actually been infected more than once. As has been stated previously, outbreaks of the disease have been known to occur in 3 or 4-year-old birds, which indicated that increased age was not the explanation of the failure of recovered fowls to again become infected. It seemed probable, therefore, that recovery from the disease was accompanied by immunity against subsequent infection. A series of tests designed to provide definite information on this point were accordingly carried out. In these tests,

birds that had recovered from infectious bronchitis at varying lengths of time after recovery were again exposed to the infection. Exposure consisted either of swabbing the trachea with a sterile cotton swab saturated with a suspension of tracheal exudate from a diseased fowl or of placing the fowls with a farm flock in which a severe outbreak of the disease was in progress. The virulence of the exudate used for the intratracheal inoculation was in all cases demonstrated by intratracheal inoculation of susceptible young cockerels with the same material. The details of the tests are given in table 3.

TABLE 3

TESTS OF THE IMMUNITY OF FOWLS THAT HAD RECOVERED FROM INFECTIOUS BRONCHITIS

Lot No.	Number of fowls	Method of exposure	Time between recovery and exposure	Results of exposure
1	7	Intratracheal inoculation	14 days	All remained healthy
2	9	Intratracheal inoculation	21-28 days	All remained healthy
3	18	Intratracheal inoculation	60 days	All remained healthy
4	58	Intratracheal inoculation	90 days	All remained healthy
5	50	Association	6 months	All remained healthy
6	250	Association	12 months	All remained healthy

ATTEMPTS TO PRODUCE IMMUNITY BY ARTIFICIAL MEANS

Since it had been demonstrated that fowls that recover from infectious bronchitis are immune to subsequent infection for a considerable period, experiments were undertaken to determine if immunity or resistance against the disease could be artificially produced. For this purpose preparations of tracheal exudate from diseased fowls were made as follows:

1. Nine grams of exudate were suspended in 90 cc of physiological saline, heated in a water bath for one hour at 55 degrees C, after which 0.5 per cent of phenol was added.
2. Six grams of exudate were suspended in 60 cc of 5 per cent formalin.
3. One gram of exudate was suspended in 9 cc of a liquid composed of equal parts of glycerine and 0.5 per cent phenolized saline.
4. One gram of exudate was suspended in 10 cc of physiological saline. Chloroform vapor was then passed through the suspension for thirty minutes.

Healthy three-months-old cockerels were given subcutaneously a 1 cc injection with each of the four preparations. These birds were later tested for immunity by intratracheal inoculation with virulent tracheal exudate from diseased fowls. The virulence of exudate used in inoculations was in all cases determined by intratracheal inoculation into susceptible cockerels. The details and results of these experiments are given in table 4.

TABLE 4

RESULTS OF EXPERIMENTS IN IMMUNIZING AGAINST INFECTIOUS BRONCHITIS BY SUBCUTANEOUS INJECTION WITH 1 CC OF TREATED TRACHEAL EXUDATE

Type of preparation	Number of fowls injected	Time between injection and inoculation	Results of inoculation
Phenolized.....	5	10 days	{ 5 showed symptoms in 24 hours 4 died within 96 hours 1 recovered 1 showed symptoms in 24 hours 3 showed symptoms in 48 hours
Formolized.....	5	10 days	{ 3 died in 96 hours 1 recovered 1 remained healthy 5 showed symptoms in 24 hours 3 died within 96 hours 2 recovered
Suspension in 50 per cent glycerine..	5	10 days	{ 5 showed symptoms in 24 hours 3 died within 96 hours 2 recovered
Treated with chloroform.....	5	10 days	{ 5 showed symptoms in 24 hours and died within 72 hours

From the results as given in table 4, it is evident that none of the preparations of tracheal exudate exerted any immunizing effect.

Further attempts to produce immunity against infectious bronchitis consisted of intratracheal injections of a small amount of varying dilutions of supernatant liquid of saline suspensions of tracheal exudate after centrifugalization. This method, instead of immunizing, caused fatal infection of all of the fowls as will be seen from the results which follow.

Ten-weeks-old cockerels were used in the first trial, there being five groups of 10 cockerels each. All the birds in each group were inoculated intratracheally with 0.1 cc of varying dilutions of the supernatant liquid. The dilutions for each group were, respectively, as follows: undiluted, 1-10, 1-100, 1-1000, 1-10,000. All of the birds sickened within 24 hours to 48 hours and died within seven days.

Day-old chicks, instead of older birds were used in the second trial, for the reason that outbreaks of the disease among chicks had, as a

rule, caused less mortality than outbreaks among older birds. This suggested that chicks might be more resistant to the infection. In the second trial, there were five groups of 10 chicks each. All the birds in each group were inoculated intratracheally with 0.1 cc of varying dilutions of the supernatant liquid. The dilutions for each group were, respectively, as follows: undiluted, 1-10, 1-100, 1-1000, 1-10,000. All the chicks sickened from infectious bronchitis within 48 hours and died within five days.

SUMMARY

1. Infectious bronchitis is a prevalent disease of fowls on poultry farms in California.

2. The disease was observed to appear suddenly, to spread rapidly, and usually to affect at least half of the fowls in a flock, causing a mortality varying from less than 5 per cent to more than 60 per cent—averaging from 10 to 20 per cent. It also caused marked decrease in the egg production of laying fowls.

3. Infectious bronchitis has occurred among fowls of all ages but it has occurred most frequently and has caused the greatest losses among fowls between the ages of four and eighteen months.

4. An attack of infectious bronchitis may cause as much loss to the poultry owner from the resultant decrease in egg production as from the death of fowls.

5. Parasitism, undernourishment and high egg production appear to be factors favoring high mortality from outbreaks of the disease.

6. Infectious bronchitis has been observed in chickens, turkeys, pigeons and ducks and in wild quail, sparrows and blackbirds.

7. Infectious bronchitis has been found to be entirely distinct from chicken-pox.

8. The principal lesions consist of hemorrhagic and mucous exudate in the lumen of the trachea and occasionally in the bronchi.

9. The disease has been definitely shown to be infectious.

10. The nature of the causative agent was not determined. Its presence could be demonstrated in the tracheal exudate of affected fowls but not in other organs.

11. The failure to associate any forms of bacteria in the etiology of the disease suggests that it may be caused by a filterable virus. Attempts to reproduce the disease by inoculation with sterile filtrates were, however, unsuccessful.

12. The disease was readily transmitted to healthy fowls by introducing into their trachea exudate from the trachea of fowls that had died from the disease. Transmission was not accomplished, however, by subcutaneous or intravenous inoculation with tracheal exudate.

13. Fowls that had recovered from the disease appeared to have an immunity which protected them against subsequent infection for at least a year.

14. Attempts to immunize fowls against the disease by subcutaneous injection of tracheal exudate which had been treated with phenol, formalin, glycerine or chloroform or by intratracheal injection of minute amounts of virulent material have been unsuccessful.

15. Treatment of diseased fowls, consisting of intratracheal injection of antiseptics not only failed to benefit the fowls, but in many instances hastened their death.

16. Vaporizing coal-tar disinfectant in poultry houses occupied by an infected flock was not found to check the spread of the disease or to reduce the mortality.

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